Green Synthesis of Silver Nanoparticles Using Plant Extracts and Their Antimicrobial Potential Against Environmental Microbiota

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Submitted By
ANNESHA DEBNATH
Registration ID: 230010367158
Enrolment ID: 2322211001036

Advisor

Samrat Chatterjee

Founder, CEO

Andragogy Life Sciences

School of Health Science and Translational Research

Sister Nivedita University

Kolkata

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This is to certify that **Ms. Annesha Debnath**, M.Sc, 4th Semester student of the Department of Microbiology, School of Health Science and Translational Research, Sister Nivedita University, Kolkata has successfully completed her four-month dissertation project titled '***Green Synthesis of Silver Nanoparticles Using Plant Extracts and Their Antimicrobial Potential Against Environmental Microbiota***' under my supervision from 5th February 2025 to 30th May 2025 at Andragogy Life Sciences, Kolkata

Samrat Chatterjee
Founder & CEO
Andragogy Life Sciences, Kolkata
Faculty, Department of Microbiology,
Gurudas College, Kolkata

ANDRAGOGY LIFE SCIENCES Kolkata-4

Samrat Chatterjee
Founder, CEO
Andragogy Life Sciences
Kolkata

4/1 Bhabanath Sen Street, Kolkata-700004
(+)91 9830134101(O)
(+)91 9903046201(P)
infochatto@gmail.com
infoandragogy@gmail.com
www.andragogylifesciences.com

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DECLARATION BY THE STUDENT

I, Annesha Debnath hereby declare that the work which is being presented in this dissertation entitled "**Green Synthesis of Silver Nanoparticles using Plant Extracts & Their Antimicrobial Potential against Environmental Microbiota**" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Microbiology is an authentic record of my own work carried out during the period from February, 2025 to May, 2025 under the supervision of Prof. Samrat Chatterjee, Department of Molecular Biology, R&D, Andragogy Life Sciences.

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Name of the Student-: Annesha Debnath

Registration no: 230010367158

Department-: Masters of Science in Microbiology

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সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

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Seal

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I would like to express my sincere gratitude to all those who have contributed to the completion of this project. Their support and assistance have been invaluable, and without their contributions, this work would not have been possible.

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Abstract

The growing interest in biologically mediated synthesis methods stems from the increasing demand for safer, non-toxic alternatives to conventional chemical approaches for nanoparticle production. In this study, a green synthesis method was employed to produce silver nanoparticles (AgNPs) using *Hibiscus rosa-sinensis* extract, serving both as a reducing and stabilizing agent. Key factors influencing the synthesis process were examined to better understand the role of plant-based compounds in nanoparticle formation.

The antimicrobial potential of the biosynthesized AgNPs was evaluated against environmental microbial isolates using an agar well diffusion assay. Microorganisms were cultured on nutrient-rich agar media, and the synthesized AgNPs were introduced to assess their inhibitory effects. A clear zone of microbial growth inhibition was seen, indicating the presence of strong antibacterial activity. In contrast, control experiments using only the plant extract exhibited no such inhibition, thereby confirming that the antimicrobial properties were specific to the silver nanoparticles.

This work highlights the effectiveness of *Hibiscus rosa-sinensis* extract as a sustainable agent in nanoparticle synthesis and underscores the potential of biologically derived AgNPs as promising antimicrobial agents in various applications.

Keywords: Green synthesis, Silver Nanoparticle, Antimicrobial activity, Plant Extract

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1.Introduction

Nanotechnology has rapidly emerged as a transformative field with significant implications across medicine, agriculture, and environmental science. Among various nanomaterials, silver nanoparticles (AgNPs) have garnered notable interest due to their unique physicochemical properties and broad-spectrum antimicrobial activity [1]. Traditionally, chemical and physical methods have been employed for the synthesis of AgNPs; however, these approaches often involve the use of toxic reagents, high energy consumption, and environmentally hazardous byproducts [2]. Consequently, there has been a growing shift toward green synthesis methods that utilize biological systems, such as plant extracts, bacteria, fungi, and algae, as eco-friendly and sustainable alternatives.

Plant-mediated synthesis of nanoparticles offers several advantages, including the natural abundance of phytochemicals, the avoidance of harsh reaction conditions, and improved biocompatibility of the final product [3]. Plant extracts serve as natural sources of reducing, capping, and stabilizing agents, which facilitate the conversion of metal ions into stable nanoparticles without the need for external chemicals. Among the various plant species explored for green nanoparticle synthesis, *Hibiscus rosa-sinensis* stands out due to its rich composition of anthocyanins, flavonoids, organic acids, and other phenolic compounds known to participate in redox reactions [4].

In this study, *Hibiscus rosa-sinensis* extract was utilized to synthesize silver nanoparticles under standard laboratory conditions. The approach aims to harness the plant's natural biochemical potential to produce AgNPs in a simple, cost-effective, and environmentally benign manner. Beyond synthesis, the study also evaluates the antimicrobial efficacy of the biologically derived AgNPs against environmental microbial isolates. Understanding the antimicrobial properties of these nanoparticles is crucial, given the rising threat of antibiotic resistance and the need for alternative antimicrobial agents [5].

Previous studies have shown that biologically synthesized AgNPs can effectively inhibit a range of bacteria and fungi by disrupting cellular membranes, generating reactive oxygen

species, and interfering with DNA replication [6]. However, the efficacy of AgNPs can vary depending on the source of synthesis and the specific biological components involved. Therefore, this research not only contributes to the expanding library of green synthesis protocols but also adds insight into the functional properties of AgNPs synthesized using *Hibiscus rosa-sinensis*, a plant with both traditional medicinal value and practical biochemical utility.

2.Review of Literature

Natural plant products are widely used today due to the increasing burden of diseases. ***Hibiscus rosa-sinensis*** Linn. (Family: Malvaceae) is a plant that is widely distributed all over the world. Its leaves, bark, roots, and flowers have been traditionally used in Indian medicine to treat a variety of pathological diseases. Numerous research studies have demonstrated that different parts of ***Hibiscus rosa-sinensis*** possess antioxidant, antimicrobial, antidiabetic, antiulcer, hepatoprotective, antifertility, antigenotoxic, and anti-inflammatory properties, which contribute to the treatment of various diseases. *Hibiscus rosa-sinensis* is also commonly included in herbal mixtures and beverages. [7]

Medicinal plants can be categorized into three major sectors: (a) modern medicine, which utilizes approximately 30 to 35 medicinal plant species; (b) organized and codified traditional medicine systems, such as Ayurveda, Siddha, Unani, Amchi, and Tibetan medicine, which are based on written treatises and make use of around 1,200 to 2,000 plant species; and (c) local health traditions, which are rooted in community practices by villagers, folk healers, vaidyas, and tribal communities, and involve the use of more than 8,000 medicinal plant species for primary healthcare [8].

Hibiscus rosa-sinensis L. is native to China. Many species of Hibiscus are cultivated for their attractive flowers. It is a shrub commonly grown in tropical regions as an ornamental plant, with various forms displaying flowers of different colours. In addition to its ornamental value, Hibiscus also possesses medicinal properties and is commonly used as a key ingredient in many herbal teas. The red-flowered variety is particularly preferred for medicinal purposes [9].

Hibiscus rosa-sinensis, commonly known as the red hibiscus, is a large shrub with various forms. It can grow in an upright form or have a broad, spreading habit. The plant can reach a height of up to 4.7 meters. All varieties of hibiscus flowers share a defining feature: the stalks of the stamens (the pollen-producing parts) and the style are fused into a long column

that protrudes from the centre of the widely spreading petals. The red variety of hibiscus produces particularly large flowers, which can grow up to 15 cm tall. Depending on the cultivated variety, the petals may be smooth or scalloped, and they can be either single or double. [10]

The origin and historical distribution of the red hibiscus is anonymous. The genus Hibiscus includes approximately 200 species, which are found throughout tropical and subtropical regions. Hibiscus is commonly cultivated as a garden ornamental from sea level up to an altitude of 500 meters. It Is sensitive to frost and may freeze during mild winters, but it typically resprouts from the base in spring. The plant grows best in full sunlight and thrives in well-drained, fertile soil that is rich in organic matter [11].

It is a well-studied plant. Reports suggest that it contains various bioactive compounds, including tannins, flavonoids, steroids, alkaloids, saponins, total phenols, total flavonoids, and total proanthocyanidins. It has been particularly noted for its high content of anthocyanins and flavonoids. Some of the major compounds identified include cyanidin-3,5-diglucoside, cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, and quercetin-3-diglucoside. Other compounds such as cyclopeptide alkaloids and additional flavonoids are also present. [12]

3.Materials and Methods

Materials

The following materials and reagents were utilized in the present study: Fresh petals of *Hibiscus rosa-sinensis*, Luria-Bertani (LB) Agar and LB Broth media, Silver nitrate ($AgNO_3$, analytical grade), Sodium citrate ($C_6H_5Na_3O_7$), Ethanol (70% v/v), Antibiotic discs: Nalidixic acid (NA), Norfloxacin (NX), Chloramphenicol (C), and Tetracycline (TE), TTC (2,3,5-triphenyltetrazolium chloride), Urea broth, Fluorescein diacetate (FDA), Hydrogen peroxide (3% H_2O_2), Sterile distilled water, saline, and laboratory-grade glassware.

3.1 Enumeration & Isolation of Microbes from Environment

3.1.1 Sample Collection

To isolate environmental microorganisms, the passive air sampling method was employed. A sterile Luria-Bertani (LB) agar plate was exposed to ambient air for 15 minutes in a semi-open environment near a balcony area, allowing airborne microbes to settle on the surface. Following exposure, the plate was incubated overnight at 37°C in a BOD (Biochemical Oxygen Demand) incubator to allow colony development.

After incubation, three morphologically distinct colonies were selected on morphological differences and designated as Sample A, B, and C. Each colony was sub-cultured individually on fresh LB agar plates using the streak plate method to obtain pure cultures. Subsequently, a single isolated colony from each pure culture plate (A, B, and C) was inoculated separately into sterile LB broth & incubated in a shaker incubator at 37°C to facilitate growth for further downstream morphological and biochemical analysis.

3.1.2 Morphological and Microscopic Analysis of Environmental Isolates

3.1.2.1 Colony Morphology and Streaking Technique:

Streaking is a fundamental technique used to isolate pure bacterial colonies from a mixed culture or to verify the purity of a sample. It helps in studying colony morphology, obtaining single colonies for further testing & ensuring a contamination-free culture.

A sterile Petri plate was divided into three sections by marking a 'Y' shape on the bottom. An inoculating loop was sterilized by flaming until red hot and then cooled. A single colony from the bacterial culture was taken using a loop.

The first section of the plate was streaked in a tight zig-zag pattern. Without reloading the loop, streaking was continued from the edge of the first section into the second section using the same, and streaking was repeated from the second to the third section. The plate was then incubated at 37°C for 24 hours to allow colony development, after which colony shape, edge, colour, and surface texture were recorded.

3.1.2.2 Gram staining

Gram characteristics is the classification of bacteria based on the structure of cell wall, determined using the Gram staining technique.

A bacterial smear was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was stained with crystal violet (primary stain) for a minute, followed by rinsing with distilled water. Iodine solution (mordant) was then applied for 1 minute and rinsed off. The slide was decolorized with alcohol or acetone for 10–30 seconds and immediately rinsed with water. Safranin (counterstain) was applied for 1 minute, followed by a final rinse and blotting to dry.

The stained slide was examined under a compound light microscope using 10X and 40X magnifications for determination morphology and Gram reaction.

3.1.2.3 Motility testing

The motility test was performed to assess the self-propelled movement of bacterial colonies**.** Three test tubes containing motility agar medium were labelled A, B, and C. Using a sterile inoculating loop, a small amount of bacterial colony from each respective culture was aseptically picked and inoculated by stabbing straight down the centre of the medium to approximately two-thirds of the depth. The inoculated tubes were incubated at 37°C for 24 hours, after which motility was assessed based on the diffusion of growth away from the stab line.

3.1.2.4 Biochemical Characterization

Biochemical characteristics refers to the metabolic & enzymatic properties used to identify & classify bacteria.

Those Biochemical Tests are -

1. Dehydrogenase Test
2. Urease Test
3. Esterase Test
4. Catalase Test

3.1.2.4.1 Dehydrogenase test - Detects the presence of dehydrogenase enzymes in microorganisms, which are involved in oxidation-reduction (redox) reactions during cellular respiration.

- Fresh air sample is taken from different locations & place in a petri plate.
- 5ml of sterile water is added to it.

- 0.2ml of 3% TTC (2,3,5-triphenyl-tetrazolium chloride) is added as substrate.
- 0.5ml of 1% glucose solution is added. The tube in 30^0C for 24hrs is incubated.
- A blank with addition of 1ml distilled water is done.
- Added 4ml methanol/sterile water after incubation & shake vigorously for 1min
- After that a test tube is kept in dark for 20 mins

Then the supernatant is filtered & the change in colour of the supernatant against the blank is noted.

3.1.2.4.2 Urease test- Detects the presence of the enzyme urease, which breaks urea. into ammonia & Carbon dioxide.

- Air sample aseptically added to the 10ml Urea broth medium & wrapped the cotton plug to avoid ammonia release.
- After that it was shaken properly & kept it in the shaker incubator for up to 24hrs.
- Observed the colour change of the medium at different time interval & compare with that of control medium without air sample.

.

3.1.2.4.3 Esterase Test - Breaks down esters (e.g., tributyrin) into fatty acids and alcohol.

- Air sample from different locations in a petri plate is taken.
- 10ml of sterile saline water is added to it.
- 0.05 ml of 200 micromolar FDA (Fluorescein diacetate) was added as substrate.
- The test tube has been for 15mins.
- The tubes at 30℃ for 24hrs is incubated.
- Run a blank with 1ml distilled water instead of air.
- Observed the colour change of the test tube against blank.

3.1.2.4.4 Catalase Test – Detects the enzyme catalase; bubbles indicate positive result.

Slide Method-

- The organism suspension from the Nutrient Broth Culture to a clean glass slide with a loop/dropper is transferred.
- 3-4 drops of 3% H_20_2 is added immediately to the culture suspension.
- The slides for any bubble formation are observed.

3.1.3 Antimicrobial Activity by Disk Diffusion Method

The disc diffusion method, also known as the Kirby-Bauer method, is a culture-based microbiology assay used to determine the antimicrobial susceptibility of bacteria. It involves placing antibiotic-impregnated paper discs on a lawn of bacteria on agar, incubating, and measuring zones of inhibition to assess the antibiotic's effectiveness.

Three Agar plates (designated as Plates A, B, and C) were prepared for antimicrobial susceptibility testing using the Disk Diffusion method. Each plate was inoculated with 50 µL of a standardized inoculum from three different unknown bacterial cultures, ensuring even distribution across the agar surface.

Antimicrobial-impregnated paper discs containing standard antibiotics—Nalidixic Acid 30µg (NA), Norfloxacin 10µg (NX), Chloramphenicol 30µg (C), and Tetracycline 30µg (TE)—were aseptically placed in four quadrants of each agar plate. The plates were then incubated at 37°C for overnight to facilitate bacterial growth and diffusion of the antibiotics through the medium. Following incubation, the diameters of the zones of inhibition around each disc were measured by using a ruler. The measured zone diameters are compared to establish standards to determine if the bacteria are susceptible, intermediate, or resistant to the antibiotics. [Ampicillin; (A) has shown susceptibility against three hence it will not be considered].

3.2 Green Synthesis of Nano-particle

3.2.1 Collection of natural ingredients which is Hibiscus petals and extraction of pigment:

Fresh Hibiscus (***Hibiscus rosa-sinensis***) flowers were collected from the garden, thoroughly washed with distilled water to remove dust and debris, and subsequently air-dried at room temperature.

A total of 2 grams of dried Hibiscus petal pieces were accurately weighed and subjected to extraction using 14 mL of 70% ethanol (v/v). The extraction process was carried out at room temperature with occasional stirring for adequate solute dissolution. Following extraction, the mixture was filtered. The filtrate was then subjected to air drying to remove excess ethanol, and the concentrated extract was incubated overnight at 37°C to ensure complete solvent evaporation and obtain a dry extract for further analysis.

3.2.2 Green Synthesis of Silver (Ag) Nanoparticles (NP) from Hibiscus plant

Silver nanoparticles (AgNPs) were biosynthesized using an ethanolic extract of ***Hibiscus rosa-sinensis*** as a reducing and stabilizing agent. Silver nitrate ($AgNO_3$) was used as the precursor for nanoparticle synthesis. For nanoparticle synthesis different concentrations of citrate buffer (0.1 M and 0.2 M) were employed to dissolve the $AgNO_3$, serving as the reaction medium. The $AgNO_3$ solutions in citrate buffer were then mixed with the Hibiscus extract under controlled conditions to facilitate the reduction of silver ions (Ag^+), leading to the formation of silver nanoparticles.

3.2.3 *Antimicrobial activity by using Silver Nano-particle*

The antibacterial activity of the biosynthesized AgNPs was assessed using the agar well diffusion method. LB agar plates inoculated with 10^6 CFU/ml of environmental isolates (A, B, and C) were prepared. Wells were bored into the agar using a sterile cork borer, and 100 µL of AgNP suspensions at concentrations of 100 µg/ml and 200 µg/ml were added into the wells. A control well containing Hibiscus extract (without silver) was also

included. Plates were incubated overnight at 37°C. Zones of inhibition were measured and compared across samples to determine dose-dependent antimicrobial efficacy. The antimicrobial activity of AgNP was assessed against environmental samples A, B, and C using the agar well diffusion method. Approximately 10^6 CFU of each microorganism were spread onto LB agar plates. Wells were prepared in the agar, and varying concentrations of AgNP combined with Hibiscus extract (100 and 200 µg/ml) were introduced into them. A control well containing only the Hibiscus extract, without AgNP, was also prepared and incubated under identical conditions. All plates were incubated overnight at 37°C. Following incubation, the plates were examined for the presence of zones of inhibition. A distinct clear zone was observed at the 200 µg/ml concentration of AgNP, indicating significant antimicrobial activity. No zone of inhibition was seen in the control well, suggesting that the antimicrobial effect was attributed specifically to the presence of AgNP.

4.Results and Discussions

4.1 Pigment content

The Hibiscus *rosa-sinensis* extract showed a vivid red coloration upon ethanol extraction, indicative of the presence of anthocyanins, particularly cyanidin derivatives. These pigments are known to contribute to the extract's reducing potential in the green synthesis of metal nanoparticles.

Figure 1 Hibiscus rosa-sinensis Extract

4.2 Synthesis of AgNP

The successful synthesis of silver nanoparticles (AgNPs) was visually confirmed by a colour change from pale yellow to brownish-red upon mixing the Hibiscus extract with $AgNO_3$. This is a classical indicator of surface plasmon resonance (SPR) in silver nanoparticles (Ahmed et al., 2016). The reaction was carried out at ambient temperature without the need for any external chemical reducers or stabilizers, further affirming the green synthesis protocol.

4.3 Effect of Silver Nitrate Concentration

Two concentrations of $AgNO_3$ (dissolved in 0.1 M and 0.2 M citrate buffer) were tested. The higher concentration led to a more intense coloration, implying a greater extent of nanoparticle formation. However, excessive ionic silver can also lead to aggregation, so optimal concentrations need to balance nanoparticle yield with stability

4.4 Isolation of environmental sample (airborne bacteria)

Environmental samples collected via open-plate exposure revealed diverse microbial colonies designated as A, B, and C. Morphological variations were observed, and further Gram staining revealed both Gram-positive and Gram-negative bacteria, indicating environmental heterogeneity in airborne microbiota.

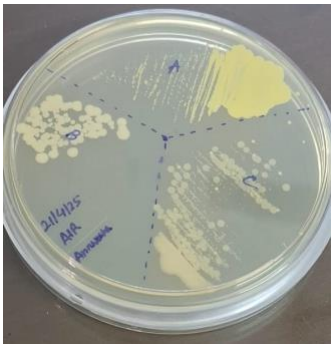


Figure 2 A, B, C (3 Types of Bacterial Cultures were inoculated)

4.5 Characterization of Selected Environmental Sample

Each bacterial isolate underwent Gram staining, motility, and biochemical tests (dehydrogenase, urease, esterase, catalase) to characterize their physiological traits.

- **Sample A** was Gram-positive, motile, and showed strong dehydrogenase and esterase activity.
- **Sample B** was Gram-negative, non-motile, and positive for urease and esterase.
- **Sample C** was non-motile, Gram-negative, and tested positive for urease and esterase but negative for dehydrogenase.

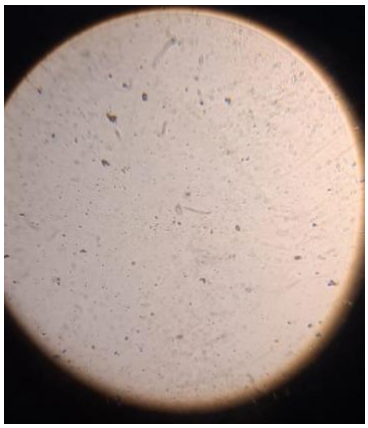


Figure 3 Gram Staining of Sample A: Gram Positive

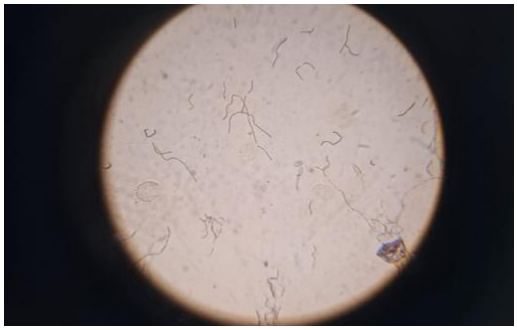


Figure 4 Gram staining of Sample B: Gram Positive

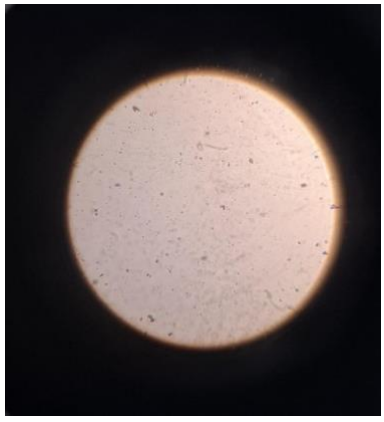


Figure 5 Gram staining of Sample C: Gram negative

Table 1 Biochemical Test Result

Biochemical Test	Sample A	Sample B	Sample C
Dehydrogenase	+ve	-ve	-ve
Esterase	+ve	+ve	+ve
Urease	-ve	+ve	+ve
Catalase	Bubble formed	No Bubbles	Bubble formed

Figure 6 Dehydrogenase Test (Control & Sample A; after 24hrs)

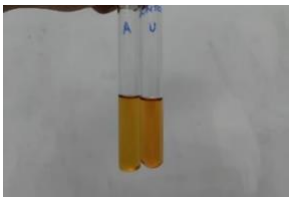


Figure 7 Urease Test (Sample A & Control)

Figure 8Esterase Test (sample A & Control)

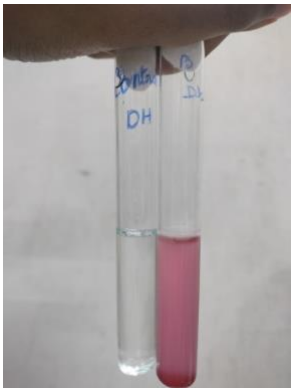


Figure 9 Dehydrogenase test (Control & Sample B)

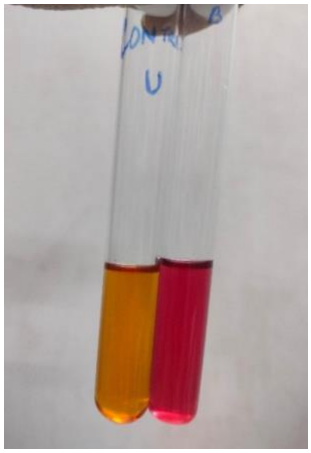


Figure 10Urease Test (Sample B & Control)

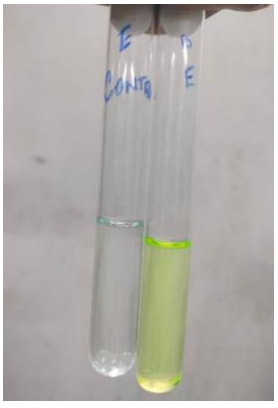


Figure 11Esterase Test (Control & Sample B)

Figure 12Dehydrogenase Test (Sample C & Control)

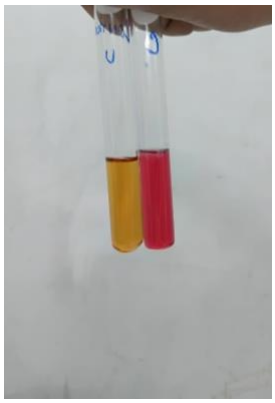


Figure 13Urease Test (Control & Sample C)

Figure 14Esterase Test (Sample C & Control)

Motility Test

Figure 15Motility Test of Sample A (Sample A is motile)

Figure 16Motility Test of Sample B (Sample B is non- motile)

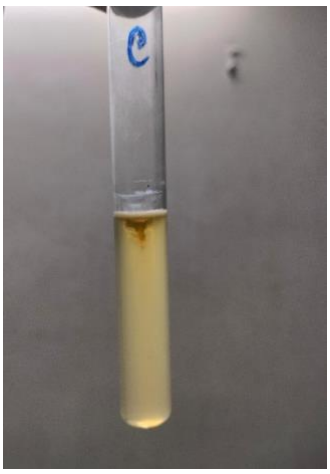


Figure 17Motility Test of Sample C (Sample C is non-motile)

4.5.1 *Antimicrobial Activity by Disk Diffusion Method*

Figure 18 Sample A: Zone of Inhibition

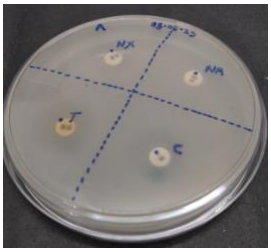


Figure 19 Sample B: Zone of Inhibition

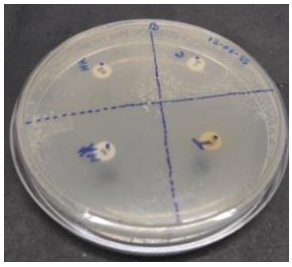
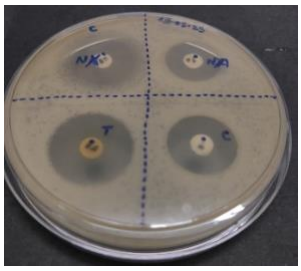


Figure 20 Sample C: Zone of Inhibition



Antibiotic (µg)	Sample A	Sample B	Sample C
Zone of Inhibition (mm)			
NA 30	X	2.1	1.76
NX 10	1.5	X	2.8
C 30	1.46	1.86	1.93
TE 30	1.8	3.06	2.26

Table 2 Antimicrobial activity by Disk Diffusion Method

4.5.2 Antibacterial activity of Hib-AgNP on Environmental sample

The antimicrobial potential of Biosynthesized Hibiscus-AgNPs was assessed using the agar well diffusion method at two concentrations: 100 µg/ml and 200 µg/ml. A clear zone of inhibition was observed only at the higher concentration (200 µg/ml), indicating dose-dependent antimicrobial activity.

- **Sample A** showed a relatively smaller zone of inhibition.
- **Sample B** exhibited the largest zone, suggesting it was the most susceptible.
- **Sample C** displayed a moderate zone, indicating moderate resistance. Control wells with only Hibiscus extract showed no inhibition, reaffirming that the antimicrobial effect was due to AgNPs alone.

Well	Sample A	Sample B	Sample C
Zone of Inhibition (mm)			
A	X	16.6	14
B	11	20.6	16.3
C	12.6	20.3	12.6
D	X	X	X

Table 3 Antibacterial activity of Hib-AgNP on Environmental Sample

These results are consistent with previous studies showing that AgNPs disrupt bacterial cell walls, generate reactive oxygen species (ROS), and interfere with replication mechanisms (7). The variability in inhibition zones reflects differences in cell wall structures, with Gram-negative bacteria generally being more susceptible due to their thinner peptidoglycan layers and outer membrane porins facilitating nanoparticle entry.

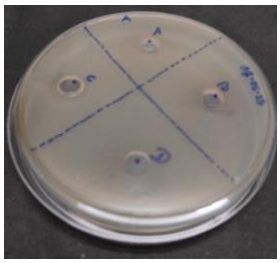


Figure 21 Zone of Inhibition of Nanoparticle (Sample A)

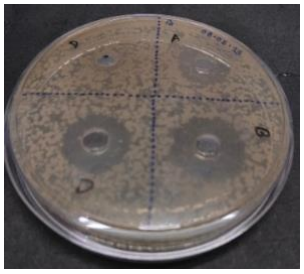


Figure 22 Zone of Inhibition of Nanoparticle (Sample B)

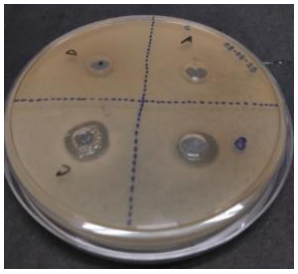


Figure 23 Zone of Inhibition of Nanoparticle (Sample C)

5. Conclusion

Hibiscus extract, when tested independently, did not exhibit any observable antimicrobial activity, as evidenced by the absence of a zone of inhibition in standard antimicrobial assays. In contrast, silver nitrate ($AgNO_3$) at a concentration of 2 mM demonstrated moderate antimicrobial activity, as indicated by the formation of a clear zone of inhibition around the application site. Interestingly, when $AgNO_3$ (2 mM) was combined with Hibiscus extract and the mixture was dissolved in citrate buffer, a significant enhancement in antimicrobial activity was observed. This synergistic effect suggests a possible interaction between the silver ions and phytochemical constituents of the Hibiscus extract, potentially leading to the formation of more bioactive silver-based complexes or nanoparticles. Furthermore, comparative analysis between two buffer concentrations revealed that 0.2 M citrate buffer facilitated more effective dissolution of the $AgNO_3$–Hibiscus extract mixture compared to 0.1 M citrate buffer. This improved solubility in the higher molarity buffer is likely responsible for the increased antimicrobial efficacy observed, possibly due to better stabilization and dispersion of active components in the medium.

6.Future Work

The current study successfully demonstrated the green synthesis of nanoparticles using eco-friendly biological extracts and established their antimicrobial activity through preliminary assays. The presence of significant zones of inhibition against selected unknown environmental bacterial samples indicate the potential of these biologically synthesized nanoparticles as effective antimicrobial agents. These promising outcomes lay the groundwork for a more comprehensive and multi-faceted exploration in the future.

Moving forward, the next phase of our research aims to delve deeper into the physicochemical characterization of these nanoparticles to better understand their morphology, stability, and surface functionalization. While initial antimicrobial assays (such as the disc diffusion method) have indicated potential efficacy, future studies will employ advanced spectroscopic and microscopic techniques to substantiate these findings. In particular, UV-Visible spectroscopy will be employed to monitor initial confirmation of nanoparticle formation and an estimation of size distribution in solution. Furthermore, Fourier-Transform Infrared Spectroscopy (FTIR) will be used to identify the functional groups involved in the reduction and stabilization of the nanoparticles. In addition to UV-Vis and FTIR, X-ray Diffraction (XRD) studies are planned to determine the crystalline nature of the nanoparticles, and Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) will be employed to observe their size, shape, and surface morphology at high resolution.

Future research will also involve testing the antimicrobial efficacy of the nanoparticles against a broader spectrum of microorganisms including multidrug-resistant strains. This will help determine whether these green-synthesized nanoparticles can be positioned as potential alternatives to conventional antibiotics. Time kill assays & Minimum Inhibitory Concentration (MIC) studies will complement the zone of inhibition tests to provide quantitative data on antimicrobial potency.

7.References

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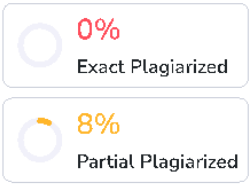
8.Plagiarism Report

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 31,2025

Plagiarized Content



Unique Content

Total Words: 699 **Total Characters:** 4477 **Plagiarized Sentences:** 3.04 **Unique Sentences:** 34.96 (92%)

Content Checked for Plagiarism

Hibiscus rosa-sinensis which is commonly known as the red hibiscus, is a large shrub with variety of forms. It can grow in an upright form or have a broad, spreading habit. The plant can reach a height of up to 4.7 meters. All varieties of hibiscus flowers share a defining feature: the stalks of the stamens (the pollen-producing parts) and the style are fused into a long column that protrudes from the centre of the widely spreading petals. The red variety of hibiscus produces particularly large flowers, which can grow up to 15 cm tall.

A sterile Petri plate was divided into three sections by marking a 'Y' shape on the bottom. An inoculating loop was sterilized by flaming until red hot and then cooled. A single colony from the bacterial culture was taken using a loop.

The first section of the plate was streaked in a tight zig-zag pattern. Without reloading the loop, streaking was continued from the edge of the first section into the second section using the same, and streaking was repeated from the second to the third section. The plate was then incubated at 37°C for 24 hours to allow colony development, after which colony shape, edge, colour, and surface texture were recorded.

Gram characteristics refer to the classification of bacteria based on the structure of their cell walls, determined using the Gram staining technique.

A bacterial smear was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was stained with crystal violet for a minute after that rinsed with distilled water. Iodine solution (mordant) was then applied for 1 minute and rinsed off. The slide was decolorized with alcohol or acetone for 10 30 seconds and immediately rinsed with water. Safranin (counterstain) was applied for 1 minute, followed by a final rinse and blotting to dry.

The stained slide was examined under a compound light microscope using 10X and 40X magnifications for determination morphology and Gram reaction.

The motility test was performed to assess the self-propelled movement of bacterial colonies.

The disc diffusion method, also known as the Kirby-Bauer method, is a culture-based microbiology assay that is used to determine the antimicrobial susceptibility of bacteria. It involves placing antibiotic-impregnated paper discs on a lawn of bacteria on agar, incubating, and measuring zones of inhibition to assess the antibiotic's effectiveness.

Silver nanoparticles (AgNPs) were biosynthesized using an ethanolic extract of Hibiscus sabdariffa as a reducing and stabilizing agent. Silver nitrate (AgNO) was used as the precursor for nanoparticle synthesis. For nanoparticle synthesis different concentrations of citrate buffer (0.1 M and 0.2 M) were employed to dissolve the AgNO , serving as the reaction medium. The AgNO solutions in citrate buffer were then mixed with the Hibiscus extract under controlled conditions to facilitate the reduction of silver ions (Ag), leading to the formation of silver nanoparticles.

The antibacterial activity of the biosynthesized AgNPs was assessed using the agar well diffusion method. LB agar plates inoculated with 10 CFU/ml of environmental isolates (A, B, and C) were prepared. Wells were bored into agar using a sterile cork borer, and 100 µL of AgNP suspensions of 100 µg/ml and 200 µg/ml were added into the wells. A control well containing Hibiscus extract (without silver) was also included. Plates were incubated overnight at 37°C. Zones of inhibition were measured and compared across samples to determine dose-dependent antimicrobial efficacy. Agar well diffusion antimicrobial assay by using AgNP
The antimicrobial activity of AgNP was assessed against environmental samples A, B, and C using the agar well diffusion method. Approximately 10 CFU of each microorganism were spread onto LB agar plates. Wells were prepared in the agar, and varying concentrations of AgNP combined with Hibiscus extract (100 and 200 g/ml) were introduced into them. A control well containing only the Hibiscus extract, without AgNP, was also prepared and incubated under identical conditions. All plates were incubated overnight at 37°C. Following incubation, the plates were examined for the presence of zones of inhibition. A distinct clear zone was observed at the 200 g/ml concentration of AgNP, indicating significant antimicrobial activity. No zone of inhibition was seen in the control well, suggesting that the antimicrobial effect was attributed specifically to the presence of AgNP.

Plagiarized Sources

The plant can reach a height of up to 4.7 meters.
https://en.wikipedia.org/wiki/Titan_arum

All varieties of hibiscus flowers share a defining feature: the stalks of the stamens (the pollen-producing parts) and the style are fused into a long column that protrudes from
https://greengardeningmatters.blogspot.com/2023/05/hibiscus-plants-with-most-beautiful.html

The motility test was performed to assess the self-propelled movement of bacterial colonies.
https://www.youtube.com/watch%3Fv%3DyvFHUzXqhcU